

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :  C12Q 1/70, C12N 5/20 C07K 13/00		A1	(11) International Publication Number: <b>WO 93/04205</b>  (43) International Publication Date: 4 March 1993 (04.03.93)
(21) International Application Number: PCT/US92/07189	(22) International Filing Date: 21 August 1992 (21.08.92)	(74) Agents: GORMAN, Edward, Hoover, Jr. et al.; Abbott Laboratories, CHAD-0377, AP6D-2, One Abbott Park Road, Abbott Park, IL 60064-3500 (US).	
(30) Priority data: 748,292 21 August 1991 (21.08.91) US		(81) Designated States: AU, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).	
(71) Applicant: ABBOTT LABORATORIES [US/US]; CHAD-0377/AP6D-2, One Abbott Park Road, Abbott Park, IL 60064-3500 (US).	(72) Inventors: MEHTA, Smriti, U. ; 1124 Kristin Drive, Libertyville, IL 60064-3500 (US). JOHNSON, Jill, E. ; 13 North Philippa, Waukegan, IL 60085 (US). DAILEY, Stephen, H. ; 472 Tyler Court, Vernon Hills, IL 60061 (US). DESAI, Suresh, M. ; 1408 Amy Lane, Libertyville, IL 60048 (US). DEVARE, Sushil, G. ; 2492 Farnsworth Lane, Northbrook, IL 60062 (US).	Published <i>With international search report.</i>	

(54) Title: MONOCLONAL ANTIBODIES TO PUTATIVE HCV E2/NS1 PROTEINS AND METHODS FOR USING SAME

(57) Abstract

Monoclonal antibodies which specifically bind to Hepatitis C Virus (HCV) E2/NS1 antigen. Also provided are hybridoma cell lines which secrete these monoclonal antibodies, methods for using these monoclonal antibodies, and assay kits for assays which contain these monoclonal antibodies.

***FOR THE PURPOSES OF INFORMATION ONLY***

**Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.**

AT	Austria	FI	Finland	MN	Mongolia
AU	Australia	FR	France	MR	Mauritania
BB	Barbados	GA	Gabon	MW	Malawi
BE	Belgium	GB	United Kingdom	NL	Netherlands
BF	Burkina Faso	GN	Guinea	NO	Norway
BG	Bulgaria	GR	Greece	NZ	New Zealand
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	PT	Portugal
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	RU	Russian Federation
CG	Congo	KP	Democratic People's Republic of Korea	SD	Sudan
CH	Switzerland	KR	Republic of Korea	SE	Sweden
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovak Republic
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CS	Czechoslovakia	LU	Luxembourg	SU	Soviet Union
CZ	Czech Republic	MC	Monaco	TD	Chad
DE	Germany	MG	Madagascar	TG	Togo
DK	Denmark	ML	Mali	UA	Ukraine
ES	Spain			US	United States of America

MONOCLONAL ANTIBODIES TO PUTATIVE HCV E2/NS1 PROTEINS  
AND METHODS FOR USING SAME

5        This application is a continuation-in-part application of pending U. S. patent applications Serial No. 07/456,162 and 07/610,180 entitled HEPATITIS C ASSAY, which enjoy common ownership and are incorporated herein by reference.

10      Background of the Invention

This invention relates generally to antibodies which specifically bind to hepatitis C virus (HCV), and more specifically, relates to a panel of novel hybridoma cells lines which secrete monoclonal antibodies which specifically bind to the putative HCV protein E2/NS1, and methods for using these 15 monoclonal antibodies.

Descriptions of hepatitis diseases causing jaundice and icterus have been known to man since antiquity. Viral hepatitis is now known to include a group of viral agents with distinctive viral organization protein structure and 20 mode of replication, causing hepatitis with different degrees of severity of hepatic damage through different routes of transmission. Acute viral hepatitis is clinically diagnosed by well-defined patient symptoms including jaundice, hepatic tenderness and an elevated level of liver transaminases such as aspartate transaminase and alanine transaminase.

25       Serological assays currently are employed to further distinguish between hepatitis-A and hepatitis-B. Non-A non-B Hepatitis (NANBH) is a term first used in 1975 that described cases of post-transfusion hepatitis not caused by either hepatitis A virus or hepatitis B virus. Feinstone et al., New 30 Engl. J. Med. 292:454-457 (1975). The diagnosis of NANBH has been made primarily by means of exclusion on the basis of serological analysis for the presence of hepatitis A and hepatitis B. NANBH is responsible for about 90% of the cases of post-transfusion hepatitis. Hollinger et al. in N. R. Rose et al., eds., Manual of Clinical Immunology, American Society for Microbiology, 35 Washington, D. C., 558-572 (1986).

Attempts to identify the NANBH virus by virtue of genomic similarity to one of the known hepatitis viruses have failed thus far, suggesting that NANBH virus has a distinctive genomic organization and structure. Fowler et

-2-

al., J. Med. Virol. 12:205-213 (1983), and Weiner et al., J. Med. Virol. 21:239-247 (1987). Progress in developing assays to detect antibodies specific for NANBH has been hampered by difficulties encountered in identifying antigens associated with the virus. Wards et al., U. S. Patent No. 4,870,076; 5 Wards et al., Proc. Natl. Acad. Sci. 83:6608-6612 (1986); Ohori et al., J. Med. Virol. 12:161-178 (1983); Bradley et al., Proc. Natl. Acad. Sci. 84:6277-6281 (1987); Akatsuka et al., J. Med. Virol. 20:43-56 (1986).

In May of 1988, a collaborative effort of Chiron Corporation with the 10 Centers for Disease Control resulted in the identification of a putative NANB agent, hepatitis C virus (HCV). M. Houghton et al. cloned and expressed in E. coli a NANB agent obtained from the infectious plasma of a chimp. Kuo et al., Science 244:359-361 (1989); Choo et al., Science 244:362-364 (1989). cDNA (copy DNA) sequences from HCV were identified which encode 15 antigens that react immunologically with antibodies present in a majority of the patients clinically diagnosed with NANBH. Based on the information available and on the molecular structure of HCV, the genetic makeup of the virus consists of single stranded linear RNA (positive strand) of molecular weight approximately 9.5 kb, and possessing one continuous translational 20 open reading frame. J. A. Cuthbert, Amer. J. Med. Sci. 299:346-355 (1990). It is a small enveloped virus resembling the Flaviviruses. Investigators have made attempts to identify the NANB agent by ultrastructural changes in hepatocytes in infected individuals. H. Gupta, Liver 8:111-115 (1988); D.W. Bradley J. Virol. Methods 10:307-319 (1985). Similar ultrastructural changes 25 in hepatocytes as well as PCR amplified HCV RNA sequences have been detected in NANBH patients as well as in chimps experimentally infected with infectious HCV plasma. T. Shimizu et al., Proc. Natl. Acad. Sci. 87:6441-6444 (1990).

30 Considerable serological evidence has been found to implicate HCV as the etiological agent for post-transfusion NANBH. H. Alter et al., N. Eng. J. Med. 321:1494-1500 (1989); Estaben et al., The Lancet: Aug. 5:294-296 (1989); C. Van Der Poel et al., The Lancet Aug. 5:297-298 (1989); G. Sbollì, J. Med. Virol. 30:230-232 (1990); M. Makris et al., The Lancet 335:1117-1119 (1990). Although the detection of HCV antibodies eliminates 70 to 80% of 35 NANBH infected blood from the blood supply system, the antibodies apparently are readily detected during the chronic state of the disease, while only 60% of the samples from the acute NANBH stage are HCV antibody positive. H. Alter et al., New Eng. J. Med. 321:1494-1500 (1989). These data

clearly indicated the need for the identification of additional HCV proteins for efficient serodiagnosis of HCV infection. Following the cloning and expression of structural protein CORE and 33C, second generation antibody assays have been developed which employ HCV CORE and 33C proteins in addition to C-100 for the detection of antibodies to HCV in NANB patients. Although the second generation assays have significantly increased the sensitivity of detection, the prolonged interval between exposure to HCV and antibody detection, and the lack of adequate information regarding the profile of immune response to various structural and non-structural proteins raises questions regarding the infectious state of the patient in the antibody negative phase during NANBH infection. Therefore, there is a need for the development of assay systems to identify acute infection to HCV and the presence of HCV.

15 Summary of the Invention

The present invention provides a panel of highly specific and novel monoclonal antibodies that can be employed for the detection of putative HCV E2/NS1 antigens. The monoclonal antibodies specifically bind to protein sequences derived from the putative HCV E2/NS1 gene. The hybridomas which produce these monoclonal antibodies are identified as follows: hybridoma H13C113 (A.T.C.C. deposit No. HB 10857) and hybridoma H23C163 (A.T.C.C. deposit No. HB 10856).

25 The specificity of these monoclonal antibodies enables the advantageous identification of HCV antigen in the putative E2/NS1 region, which identification can be useful in differentiation studies as well as in the diagnosis and evaluation of HCV (NANB) infections.

30 In a preferred assay format, a test sample which may contain HCV antigens is contacted with a solid phase to which a polyclonal or a monoclonal anti-HCV E2/NS1 antibody or a fragment thereof has been bound, to form a mixture. This mixture is incubated for a time and under conditions sufficient for antigen/antibody complexes to form. The so-formed complexes then are contacted with an indicator reagent comprising a 35 monoclonal or polyclonal antibody or a fragment thereof, specific for the HCV antigen attached to a signal generating compound to form a second mixture. This second mixture is reacted for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence of HCV antigen is determined by detecting the measurable signal generated. The amount of

HCV present in the test sample, thus the amount of HCV antigen captured on the solid phase, is proportional to the amount of signal generated.

Alternatively, an indicator reagent comprising a monoclonal or polyclonal antibody, or fragment thereof, specific for HCV E2/NS1 antigen and a signal generating compound is added to a polyclonal or monoclonal anti-HCV antibody or fragment thereof coated on a solid phase and the test sample, to form a mixture. This mixture is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence and amount of HCV present in the test sample, and thus the amount of HCV antigen captured on the solid phase, is determined by detecting the measurable signal. The amount of HCV present in the test sample is proportional to the amount of signal generated.

In another alternate assay format, one or a combination of more than one monoclonal antibody of the invention can be employed as a competitive probe for the detection of antibodies to HCV E2/NS1 antigen. For example, HCV E2/NS1 antigens, either alone or in combination, can be coated on a solid phase. A test sample suspected of containing antibody to HCV E2/NS1 antigen then is incubated with an indicator reagent comprising a signal generating compound and a monoclonal antibody of the invention for a time and under conditions sufficient to form antigen/antibody complexes of either the test sample and indicator reagent to the solid phase or the indicator reagent to the solid phase. The reduction in binding of the monoclonal antibody to the solid phase can be quantitatively measured. A measurable reduction in the signal compared to the signal generated from a confirmed negative NANBH test sample would indicate the presence of anti-HCV E2/NS1 antibody in the test sample.

In yet another assay format, a test sample is contacted with a solid phase to which HCV E2/NS1 proteins are attached and an indicator reagent comprising a monoclonal antibody or fragment thereof specific for HCV E2/NS1 attached to a signal generating compound, to form a mixture. The mixture is incubated for a time and under conditions sufficient for antibody/antigen complexes to form. The presence of anti-HCV present in the test sample is determined by detecting the measurable signal generated, and comparing the signal to the measured signal generated from a known negative sample. A measurable reduction of signal of the test sample, compared to the known negative sample's signal, is indicative of the

-5-

presence of anti-HCV antibodies. Competitive assays for the detection of anti-HCV antibody using antigens free in solution also can be performed.

The presence of HCV E2/NS1 antigen can be detected in a tissue  
5 sample by contacting the tissue sample with an indicator reagent comprising  
a signal generating compound attached to a monoclonal antibody which  
specifically binds to HCV E2/NS1 antigen or fragment thereof, to form a  
mixture. This mixture is incubated for a time and under conditions sufficient  
for antigen/antibody complex to form. The presence of HCV E2/NS1 antigen  
10 present in the tissue sample is determined by detecting the signal generated.

Also provided are kits useful for determining the presence of HCV NS1  
antigen or antibody in test samples that include the monoclonal antibodies of  
the invention.

15

#### Brief Description of the Drawings

FIG. 1 is an illustration of the location of the recombinant HCV proteins  
on the HCV genome employed either as immunogens for the generation of  
monoclonal antibodies or for their characterization.

20

FIG. 2 is a Western blot analysis illustrating specific binding of  
monoclonal antibodies H13C113 and H23C163 to HCV NS1.

25

FIG. 3 is a profile of PEPSCAN analysis with overlapping hexamer  
peptides (a.a. 600-720 of HCV) of monoclonal antibody H13C113 illustrating  
the epitope specificity of H13C13 to HCV a.a. 649-655.

30

FIG. 4 is a profile of PEPSCAN analysis with overlapping hexamer  
peptides (a.a. 600-720 of HCV) of monoclonal antibody H23C163 illustrating  
the epitope specificity of H13C13 to HCV a.a. 649-655.

#### Detailed Description of the Invention

The present invention provides novel monoclonal antibodies to the  
putative HCV E2/NS1 protein, methods for using the monoclonal antibodies,  
35 and kits which contain these monoclonal antibodies.

The monoclonal antibodies of the present invention can be employed  
in various assay systems to determine the presence, if any, of HCV E2/NS1  
proteins in a test sample. Fragments of these monoclonal antibodies

-6-

provided also may be used. For example, in a first assay format, a polyclonal or monoclonal anti-HCV E2/NS1 antibody or fragment thereof, or a combination of these antibodies, which has been coated on a solid phase, is contacted with a test sample which may contain HCV E1/NS1 proteins, to 5 form a mixture. This mixture is incubated for a time and under conditions sufficient to form antigen/antibody complexes. Then, an indicator reagent comprising a monoclonal or a polyclonal antibody or a fragment thereof, which specifically binds to the HCV E2/NS1 region, or a combination of these antibodies, to which a signal generating compound has been attached, is 10 contacted with the antigen/antibody complexes to form a second mixture. This second mixture then is incubated for a time and under conditions sufficient to form antibody/antigen/antibody complexes. The presence of HCV E2/NS1 antigen present in the test sample and captured on the solid phase, if any, is determined by detecting the measurable signal generated by the 15 signal generating compound. The amount of HCV E2/NS1 antigen present in the test sample is proportional to the signal generated.

Alternatively, a polyclonal or monoclonal anti-HCV E2/NS1 antibody or fragment thereof, or a combination of these antibodies which is bound to a 20 solid support, the test sample and an indicator reagent comprising a monoclonal or polyclonal antibody or fragments thereof, which specifically binds to HCV E2/NS1 antigen, or a combination of these antibodies to which a signal generating compound is attached, are contacted to form a mixture. This mixture is incubated for a time and under conditions sufficient to form 25 antibody/antigen/antibody complexes. The presence, if any, of HCV E2/NS1 proteins present in the test sample and captured on the solid phase is determined by detecting the measurable signal generated by the signal generating compound. The amount of HCV proteins present in the test sample is proportional to the signal generated.

30

In another alternate assay format, one or a combination of one or more monoclonal antibodies of the invention can be employed as a competitive probe for the detection of antibodies to HCV protein. For example, HCV proteins, either alone or in combination, can be coated on a solid phase. A 35 test sample suspected of containing antibody to HCV E2/NS1 antigen then is incubated with an indicator reagent comprising a signal generating compound and at least one monoclonal antibody of the invention for a time and under conditions sufficient to form antigen/antibody complexes of either the test sample and indicator reagent to the solid phase or the indicator

reagent to the solid phase. The reduction in binding of the monoclonal antibody to the solid phase can be quantitatively measured. A measurable reduction in the signal compared to the signal generated from a confirmed negative NANBH test sample indicates the presence of anti-HCV E2/NS1 antibody in the test sample.

10 In yet another detection method, each of the monoclonal antibodies of the present invention can be employed in the detection of HCV antigens in fixed tissue sections, as well as fixed cells by immunohistochemical analysis.

15 In addition, these monoclonal antibodies can be bound to matrices similar to CNBr-activated Sepharose and used for the affinity purification of specific HCV proteins from cell cultures, or biological tissues such as blood and liver.

20 The monoclonal antibodies of the invention can also be used for the generation of chimeric antibodies for therapeutic use, or other similar applications.

25 The monoclonal antibodies or fragments thereof can be provided individually to detect HCV E2/NS1 antigens. It is contemplated that combinations of the monoclonal antibodies (and fragments thereof) provided herein also may be used together as components in a mixture or "cocktail" of at least one anti-HCV E2/NS1 antibody of the invention with antibodies to other HCV regions, each having different binding specificities. Thus, this cocktail can include the monoclonal antibodies of the invention which are directed to HCV E2/NS1 proteins and other monoclonal antibodies to other antigenic determinants of the HCV genome. Examples of other monoclonal antibodies useful for these contemplated cocktails include those to HCV C-30 100, HCV 33C, HCV CORE, HCV NS5 and/or HCV putative ENV, which are disclosed in, for example, U. S. Serial No. 07/610,175 entitled MONOCLONAL ANTIBODIES TO HEPATITIS C VIRUS AND METHOD FOR USING SAME, U.S.S.N. 07/610,175 entitled MONOCLONAL ANTIBODIES TO HCV 33C PROTEINS AND METHODS FOR USING SAME, U.S. Serial No. 07/648,475 entitled MONOCLONAL ANTIBODIES TO PUTATIVE HCV ENVELOPE REGION AND METHODS FOR USING SAME, U.S. Serial No. 07/648,473 entitled MONOCLONAL ANTIBODIES TO HCV CORE PROTEINS AND METHODS FOR USING SAME and in co-filed patent application entitled MONOCLONAL ANTIBODIES TO HCV NS5 PROTEIN AND METHODS FOR

USING SAME, U.S. Serial No. 07/748,563, all of which enjoy common ownership and are incorporated herein by reference. This cocktail of monoclonal antibodies as described herein would be used in the assay formats detailed herein in place of the monoclonal antibody to HCV E2/NS1, 5 and thus would be able to detect the E2/NS1 and other HCV antigens.

The polyclonal antibody or fragment thereof which can be used in the assay formats should specifically bind to HCV putative E2/NS1 region or other HCV proteins used in the assay, such as HCV C-100 protein, HCV 33C 10 protein, HCV CORE, HCV ENV or HCV NS5 protein. The polyclonal antibody used preferably is of mammalian origin; human, goat, rabbit or sheep anti-HCV polyclonal antibody can be used. Most preferably, the polyclonal antibody is rabbit polyclonal anti-HCV antibody. The polyclonal antibodies used in the assays can be used either alone or as a cocktail of polyclonal 15 antibodies. Since the cocktails used in the assay formats are comprised of either monoclonal antibodies or polyclonal antibodies having different HCV specificity, they would be useful for diagnosis, evaluation and prognosis of HCV infection, as well as for studying HCV protein differentiation and specificity.

20

Test samples which can be tested by the methods of the present invention described herein include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, urine, biological fluids such as cell culture supernatants, fixed tissue specimens and fixed cell specimens.

25

The "solid phase" is not critical and can be selected by one skilled in the art. Thus, latex particles, microparticles, magnetic or non-magnetic beads, membranes, plastic tubes, walls of microtiter wells, glass or silicon chips and sheep red blood cells are all suitable examples. Suitable methods for 30 immobilizing peptides on solid phases include ionic, hydrophobic, covalent interactions and the like. A "solid phase", as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the solid phase can retain an 35 additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized

-9-

upon (attached to) the solid phase and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid phase material before the performance of the assay or during the performance of the assay.

5 The solid phase thus can be a plastic, derivatized plastic, magnetic or non-magnetic metal, glass or silicon surface of a test tube, microtiter well, sheet, bead, microparticle, chip, and other configurations known to those of ordinary skill in the art.

10 It is contemplated and within the scope of the invention that the solid phase also can comprise any suitable porous material with sufficient porosity to allow access by detection antibodies and a suitable surface affinity to bind antigens. Microporous structures are generally preferred, but materials with gel structure in the hydrated state may be used as well. Such useful solid supports include:

15 natural polymeric carbohydrates and their synthetically modified, cross-linked or substituted derivatives, such as agar, agarose, cross-linked alginic acid, substituted and cross-linked guar gums, cellulose esters, especially with nitric acid and carboxylic acids, mixed cellulose esters, and cellulose ethers;

20 natural polymers containing nitrogen, such as proteins and derivatives, including cross-linked or modified gelatins;

25 natural hydrocarbon polymers, such as latex and rubber;

30 synthetic polymers which may be prepared with suitably porous structures, such as vinyl polymers, including polyethylene, polypropylene, polystyrene, polyvinylchloride, polyvinylacetate and its partially hydrolyzed derivatives, polyacrylamides, polymethacrylates, copolymers and terpolymers of the above polycondensates, such as polyesters, polyamides, and other polymers, such as polyurethanes or polyepoxides;

35 porous inorganic materials such as sulfates or carbonates of alkaline earth metals and magnesium, including barium sulfate, calcium sulfate, calcium carbonate, silicates of alkali and alkaline earth metals, aluminum and magnesium; and aluminum or silicon oxides or hydrates, such as clays, alumina, talc, kaolin, zeolite, silica gel, or glass (these materials may be used as filters with the above polymeric materials); and

-10-

mixtures or copolymers of the above classes, such as graft copolymers obtained by initializing polymerization of synthetic polymers on a pre-existing natural polymer. All of these materials may be used in suitable shapes, such as films, sheets, or plates, or they may be coated onto or bonded or laminated to appropriate inert carriers, such as paper, glass, plastic films, or fabrics.

10 The porous structure of nitrocellulose has excellent absorption and adsorption qualities for a wide variety of reagents including monoclonal antibodies. Nylon also possesses similar characteristics and also is suitable.

15 It is contemplated that such porous solid supports described hereinabove are preferably in the form of sheets of thickness from about 0.01 to 0.5 mm, preferably about 0.1 mm. The pore size may vary within wide limits, and is preferably from about 0.025 to 15 microns, especially from about 20 0.15 to 15 microns. The surfaces of such supports may be activated by chemical processes which cause covalent linkage of the antigen or antibody to the support. The irreversible binding of the antigen or antibody is obtained, however, in general, by adsorption on the porous material by poorly understood hydrophobic forces. Suitable solid supports also are described in U.S. Patent Application Serial No. 227,272.

25 The indicator reagent comprises a signal generating compound (label) which is capable of generating a measurable signal detectable by external means conjugated (attached) to a specific binding member for HCV. "Specific binding member" as used herein means a member of a specific binding pair. That is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. In addition to being an antibody member of a specific binding pair for HCV, the indicator reagent also can be a member of any specific binding pair, including either 30 hapten-anti-hapten systems such as biotin or anti-biotin, avidin or biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor molecule, an enzyme cofactor and an enzyme, an enzyme inhibitor or an enzyme, and the like. An immunoreactive specific binding member can be an antibody, an antigen, or an antibody/antigen complex that 35 is capable of binding either to HCV as in a sandwich assay, to the capture reagent as in a competitive assay, or to the ancillary specific binding member as in an indirect assay.

-11-

The various signal generating compounds (labels) contemplated include chromogens, catalysts such as enzymes, luminescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as acridinium, phenanthridinium and dioxetane compounds, radioactive elements, and direct visual labels. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances.

10

Other embodiments which utilize various other solid phases also are contemplated and are within the scope of this invention. For example, ion capture procedures for immobilizing an immobilizable reaction complex with a negatively charged polymer, described in co-pending U. S. Patent 15 Application Serial No. 150,278 corresponding to EP publication 0326100, and U. S. Patent Application Serial No. 375,029 (EP publication no. 0406473) both of which enjoy common ownership and are incorporated herein by reference, can be employed according to the present invention to effect a fast solution-phase immunochemical reaction. An immobilizable immune complex is separated from the rest of the reaction mixture by ionic interactions between the negatively charged poly-anion/immune complex and the previously treated, positively charged porous matrix and detected by using various signal generating systems previously described, including those described in chemiluminescent signal measurements as described in co-pending U.S. Patent Application Serial No. 921,979 corresponding to EPO Publication No. 0 273,115, which enjoys common ownership and which is incorporated herein by reference.

Also, the methods of the present invention can be adapted for use in systems which utilize microparticle technology including in automated and semi-automated systems wherein the solid phase comprises a microparticle. Such systems include those described in pending U. S. Patent Applications 425,651 and 425,643, which correspond to published EPO applications Nos. EP 0 425 633 and EP 0 424 634, respectively, which are incorporated herein by reference.

The use of scanning probe microscopy (SPM) for immunoassays also is a technology to which the monoclonal antibodies of the present invention are easily adaptable. In scanning probe microscopy, in particular in atomic

-12-

force microscopy, the capture phase, for example, at least one of the monoclonal antibodies of the invention, is adhered to a solid phase and a scanning probe microscope is utilized to detect antigen/antibody complexes which may be present on the surface of the solid phase. The use of scanning tunnelling microscopy eliminates the need for labels which normally must be utilized in many immunoassay systems to detect antigen/antibody complexes. Such a system is described in pending U. S. patent application Serial No. 662,147, which enjoys common ownership and is incorporated herein by reference.

10 The use of SPM to monitor specific binding reactions can occur in many ways. In one embodiment, one member of a specific binding partner (the analyte specific substance, which is the monoclonal antibody of the invention) is attached to a surface suitable for scanning. The attachment of the analyte specific substance may be by adsorption to a test piece which comprises a solid phase of a plastic or metal surface, following methods known to those of ordinary skill in the art. Or, covalent attachment of a specific binding partner (analyte specific substance) to a test piece which test piece comprises a solid phase of derivatized plastic, metal, silicon, or glass may be utilized. Covalent attachment methods are known to those skilled in the art and include a variety of means to irreversibly link specific binding partners to the test piece. If the test piece is silicon or glass, the surface must be activated prior to attaching the specific binding partner. Activated silane compounds such as triethoxy amino propyl silane (available from Sigma Chemical Co., St. Louis, MO), triethoxy vinyl silane (Aldrich Chemical Co., Milwaukee, WI), and (3-mercaptopropyl)-trimethoxy silane (Sigma Chemical Co., St. Louis, MO) can be used to introduce reactive groups such as amino-, vinyl, and thiol, respectively. Such activated surfaces can be used to link the binding partner directly (in the cases of amino or thiol) or the activated surface can be further reacted with linkers such as glutaraldehyde, bis (succinimidyl) suberate, SPPD 9 succinimidyl 3-[2-pyridylidithio] propionate), SMCC (succinimidyl-4-[N-maleimidomethyl] cyclohexane-1-carboxylate), SIAB (succinimidyl [4-iodoacetyl] aminobenzoate), and SMPB (succinimidyl 4-[1-maleimidophenyl] butyrate) to separate the binding partner from the surface.

15 20 25 30 35 The vinyl group can be oxidized to provide a means for covalent attachment. It also can be used as an anchor for the polymerization of various polymers such as poly acrylic acid, which can provide multiple attachment points for specific binding partners. The amino surface can be reacted with oxidized dextrans of various molecular weights to provide hydrophilic linkers of

different size and capacity. Examples of oxidizable dextrans include Dextran T-40 (molecular weight 40,000 daltons), Dextran T-110 (molecular weight 110,000 daltons), Dextran T-500 (molecular weight 500,000 daltons), Dextran T-2M (molecular weight 2,000,000 daltons) (all of which are available from

5      Pharmacia, Piscataway, N.J.), or Ficoll (molecular weight 70,000 daltons (available from Sigma Chemical Co., St. Louis, MO). Also, polyelectrolyte interactions may be used to immobilize a specific binding partner on a surface of a test piece by using techniques and chemistries described by pending U. S. Patent applications Serial No. 150,278, filed January 29, 1988, and Serial

10     No. 375,029, filed July 7, 1989, each of which enjoys common ownership and each of which is incorporated herein by reference. The preferred method of attachment is by covalent means. Following attachment of a specific binding member, the surface may be further treated with materials such as serum, proteins, or other blocking agents to minimize non-specific binding. The

15     surface also may be scanned either at the site of manufacture or point of use to verify its suitability for assay purposes. The scanning process is not anticipated to alter the specific binding properties of the test piece.

While the present invention discloses the preference for the use of solid phases, it is contemplated that the monoclonal antibodies of the present invention can be utilized in non-solid phase assay systems. These assay systems are known to those skilled in the art, and are considered to be within the scope of the present invention.

25       It is contemplated that the reagent employed for the assay can be provided in the form of a kit with one or more containers such as vials or bottles, with each container containing a separate reagent such as a monoclonal antibody, or a cocktail of monoclonal antibodies, detection reagents and washing reagents employed in the assay.

30       The following examples demonstrate the advantages and utility of this invention for serodiagnosis of HCV by describing methods for the development, characterization, epitope mapping and clinical utility of these monoclonal antibodies. The methods used for monoclonal antibody

35     development follow procedures known in the art and detailed on Kohler and Milstein, Nature 256:494 (1975) and reviewed in J.G.R. Hurrel, ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boco Ratan, FL (1982). Another method of monoclonal antibody development which is based on the Kohler and Milstein method is that of L. T.

-14-

Mimms et al., Virology 176:604-619 (1990), which is incorporated herein by reference. These examples are meant to illustrate, but not to limit, the spirit and scope of the invention.

5

## EXAMPLES

EXAMPLE 1Immunization of Mice with SEQ. ID. No. 6Selection of Synthetic Peptide for Generation of Monoclonal Antibodies to  
10 HCV E2/NS1 Region

15 Immunogenic domains of E2/NS1region of HCV genome encompassing a.a.600-720 (SEQ. ID. NO. 1) were mapped with PEPSCAN analysis. A PEPSCAN kit was purchased from Cambridge Research Bioscience (Valley Stream, New York, U.S.A.) to synthesize a series of overlapping hexamer peptides (overlap of five amino acids) encompassing HCV a.a. 600-720 (SEQ. ID. NO. 1), on derivitized polypropylene pins supplied by the manufacturer. The synthesis protocol supplied with the kit was followed exactly for the synthesis of these peptides. Briefly, the polypropylene pins which contained the *F-moc*  $\beta$ -alnine as the end group 20 amino acid were deprotected with 20% (v/v) piperidine in dimethylformamide (DMF) for 30 min. Pins were washed with DMF (1x5 min.), Methanol (4x2 min.) followed by a final DMF wash (1x5 min.). *F-moc* active esters of amino acids were prepared at 30 mM concentration in 1-hydroxybenzotriazole (HOBt) in DMF. Amino acids were dispensed (175 $\mu$ l) in wells of 96 well 25 microtiter plates supplied with the kit in desired sequence, starting at the carboxy terminus. Deprotected pins were lowered in the amino acid solutions and incubated at room temperature ( RT ) overnight. Following the DMF methanol wash sequence as described above, the deprotection, washing and coupling steps were repeated until all amino acid in each of the peptides 30 sequence were coupled. After a final deprotection step, the terminal amino acids were acetylated by incubating the pins with DMF:acetic anhydride:triethylamine at 5:2:1 (v/v/v) for 90 min. at RT. Following the DMF/methanol wash sequence, pins were air dried. Before the serological analysis, the final side chain deprotection and neutralization was 35 accomplished by treating the pins with Trifluoroacetic acid:Phenol:Ethanedithiol at 95:2.5:2.5 (v/w/v). Pins were washed with dichloromethane (2x2 min.), 5% diisopropylethylamine/ dichloromethane (1x5 min.) and dichloromethane (1x5 min.). Finally, pins were air dried, washed

-15-

with water, soaked in methanol for 18 hrs., dried and stored dessicated in refrigerator.

FAB dimers of IgG purified from sera of individuals seropositive for antibodies to HCV proteins were used as the primary antibody to for the serological analysis of these peptides using the EIA procedure recommended by the manufacturer . Briefly, the primary antibody was diluted to appropriate concentration in phosphate buffered saline ( PBS ) containing 0.1% Tween-20® ( Bio-Rad, Richmond, CA), 1% ovalbumin (available from Sigma, St. Louis, MO.), and 1% bovine serum albumin (available from Sigma). Peptide pins were incubated with the primary antibody overnight at 4°C. Following several washes with PBS/Tween-20®, pins were incubated with appropriately diluted goat anti-mouse HRPO for 1 hr. at room temperature. Azido-di-3-ethyl-benzthiazodinsulphonate dissolved in a phosphate-citrate buffer containing hydrogen peroxide was used as the color developing reagent. The optical density of the color developed was measured at 405 nm after incubation of the pins with the developing reagent for 15-20 min. Based on the reactivity of these sera in EIA, four amino acid sequences (a.a. 607-627 (SEQ. ID. No. 2), a.a. 643-663 (SEQ. ID. No. 3), a.a.666-683 (SEQ. ID. No. 4) and a.a.671-691 (SEQ. ID NO. 5 were identified as the immunogenic domains as disclosed in U.S.patent application no. 610,180 previously incorporated herein by reference. Each of these four sequences and an additional sequence, which was the combination of the two most immunogenic sequences ( a.a. 643-683 ) (SEQ. ID. NO. 6) were synthesized by a stepwise solid phase synthesis starting at the carboxy terminus by a procedure similar to that described in E.Gross and T.Heinbofer, eds. Barany and Merrifield, *The Peptides* 2:1284, Academic Press, New York, New York. Based on the EIA reactivity of a panel of HCV positive sera as disclosed in the U.S.patent application no. 610,180 previously incorporated herein by reference, peptide 643-683 (SEQ. ID NO. 6) was chosen as the immunogen for the generation of monoclonal antibodies to HCV NS1. FIG. 1 shows the location of these peptides on the HCV genome.

#### Immunization of Mice

Female Balb/c mice were immunized with approximately 50 ug of the crude peptide 643-683 ( HCV a.a. 643-683, SEQ. ID. NO. 6) using the RIBI adjuvant system ( RIBI Immunochemicals Res., U.S.A.). On day one, mice received 50 ug of the peptide with 50 ug each of Trehalose dimycolate (TDM) and *M.Phlei* in a buffer emulsion prepared according to the manufacturer's

-16-

instructions. Subsequent immunizations were done on day 18, 34, 42 and 63. Mice were bled on day 25 and 77, and the immune response was assessed by EIA using microtiter plates coated with the immunogen. Mice were allowed to rest for at least eight weeks before the fusion.

5

#### Enzyme-Linked Immunoassay (EIA)

The immune response to the immunizing antigen was assessed by microtiter EIA. Wells of microtiter plates were coated with 100  $\mu$ l of purified synthetic peptide (a.a. 643-683, SEQ. ID. NO. 6) on 0.1M bicarbonate buffer at pH 9.5. After washing with Phosphate Buffered Saline (PBS) which also contained 0.01% sodium dodecyl sulfate (SDS) and 0.05% Tween-20® (available from Bio-Rad Laboratories, Richmond, CA), free sites were overcoated with 1% BSA in bicarbonate buffer at pH 9.5. Plates were stored at 4°C following a final wash. Sera from native or immunized mice were serially diluted in 100  $\mu$ l of dilution buffer which contained 20 mM sodium phosphate, pH 7.4, 0.15 M NaCl, 20% normal goat serum, 10% fetal calf serum, 5 mM EDTA, 10 mM EGTA, 50 mM Tris, 0.2% Tween-20®, with sodium azide as a preservative (at pH 6.8). The diluted sera were reacted with the antigen for three (3) hours at 37°C. The plates were washed and 100  $\mu$ l of appropriately diluted goat anti-mouse IgG (heavy [h] and light [l] chain) Horseradish Peroxidase (HRPO)-conjugated antibody (Jackson Immunochemicals, West Grove, PA) was added. The plates were incubated at 37°C for two (2) hours. After a final wash, 100  $\mu$ l of o-phenylenediamine:2 HCl (OPD) color reagent was added. The reaction was carried out at room temperature for 10 to 30 minutes, and then stopped by the addition of 1 ml of 1 N H<sub>2</sub>SO<sub>4</sub>. The absorbance at 492/600 nm was recorded, which was found to be directly proportional to the amount of specific antibody bound to the antigen.

30

#### EXAMPLE 2

##### Cell Fusion

Upon demonstration of specific anti-HCV antibody present at reasonable titers in sera of immunized mice, the mice were allowed to rest for at least eight weeks prior to a pre-fusion boost of antigen. The pre-fusion antigen boost then was performed by intravenous (IV) tail vein injection of approximately 40  $\mu$ g of respective purified HCV synthetic peptide (SEQ. ID. NO. 6). Three days later the mice were sacrificed, and their spleens which contained anti-HCV antibody-producing cells were disrupted into single cells. These single cell suspensions were treated with 0.83% NH<sub>4</sub>Cl to remove red

blood cells, and then these suspensions were mixed with SP2/0 cells at a 10:1 (SP2/0:spleen cells) ratio. The mixed cells were centrifuged, washed once with serum-free medium, and again centrifuged. The fusogen polyethylene glycol (PEG) was used to form hybrids of the immune donor

5 spleen cells with the myeloma cell line SP2/0 (HRPT neg.). Kohler and Milstein, Nature 356:494 (1975), and reviewed in J.G.R. Hurrel, ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boco Ratan, FL (1982). Briefly, fusion of the spleen and SP2/0 cells was accomplished by exposing the pellet to 40% PEG (ATCC, mw 1300-1600) in serum-free Iscoe's Modified Dulbecco's Medium (IMDM) for two minutes. The PEG and cell suspension was diluted slowly by the addition of 20 ml of serum-free IMDM over a period of five minutes, followed by collection of the cells by centrifugation. The supernatant was decanted and replaced with 30 ml IMDM containing 20% fetal bovine serum (FBS) (Hyclone

10 Laboratories, Logan, Utah) with HAT (hypoxanthene, aminopterin and thymidine) media in order to select for hybridomas. Spleen cells from one non-immune BABB/c mouse also were added as a feeder layer. The cells were plated at 0.1 ml/well in three 96-well tissue culture plates. An additional 0.1 ml of HAT media was added to each well three days later. At weekly

15 intervals thereafter, one-half the media was replaced with IMDM containing 20% FBS with HT (hypoxanthene and thymidine), and hybrids were allowed to grow for an additional seven to fourteen days.

It was found that some of the hybrids were composed of spleen cells

25 making antibody to HCV fused with SP2/0 cells. Briefly, the fusogen promoted fusion of spleen cell and SP2/0 cell membranes, which formed a heterokaryon containing nuclei of both cells. Eventually, the dissimilar nuclei fuse produced a single nucleus capable of synchronous mitosis. As the fused cells divided, the hybrid stabilized by losing chromosomes of each nucleus.

30 The fused cells were plated into multiple 96-well plates at  $10^5$  to  $10^6$  cells per well. The hybrid cells formed from SP2/0:spleen cell fusions were selectively propagated by culturing in HAT medium. All unused SP2/0 or SP2/0:SP2/0 fused cells were prevented from growing aminopterin, and unfused spleen cells on spleen:spleen fused cells died off in culture. Only SP2/0:spleen cell

35 hybrids grew in the HAT selective medium.

-18-

EXAMPLE 3

Screening and Cloning of Monoclonal Antibodies

After 10 to 14 days, culture fluids from wells containing hybridoma cell growth were screened for the presence of a monospecific antibody as follows.

- 5 Each of the hybridoma supernatants from the NS1 fusions were tested by the EIA procedure described in Example 1 with the synthetic peptide a.a. 643-683 (SEQ. ID. NO. 6) coated on the solid phase. Hybridoma culture fluids reacting specifically to the immunogen, i.e., HCV protein SEQ. ID. NO. 6 were selected.
- 10 for cloning by the limiting dilution method, using the guidelines outlines by J. W. Goding, Monoclonal Antibodies: Principles and Practices, Academic Press, New York (1983). Culture supernatant of cloned samples were tested again by EIA with the immunogen as described above in Example 1, for the confirmation of monospecific reactivity to HCV protein sequence. Clones with strongest reactivity specifically to the synthetic
- 15 peptide were selected for expansion and further analysis.

EXAMPLE 4

Amplification of Antibody Yields by Ascites Method

In order to obtain greater amounts of monoclonal antibodies, 10 to 20 million cloned cells of the desired hybridoma cell line were inoculated into a BALB/c mouse previously treated i.p. with 0.5 ml pristane (2,6,10,14-tetramethylpentadecane) by the method outlined in J.G.R. Hurrel, ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boco Ratan, FL (1982). Pristane treatment enhanced growth of mouse myeloma hybrids within the peritoneum of the mouse, and the ascites fluids which formed were rich in the monoclonal antibody secreted by the hybrid cells. After formation of adequate ascites fluid (approximately seven days), the mice were sacrificed and the ascites were withdrawn from the peritoneum, clarified by centrifugation and stored at -20°C. Monoclonal antibodies from ascites fluid were purified using protein-A sepharose (according to J.G.R. Hurrell ed., supra). All characterization procedures described herein were performed with either culture supernatants, ascites fluids or protein-A purified IgG.

35

EXAMPLE 5

Characterization of Monoclonal Antibodies

EIA

Purified IgG of monoclonal antibodies were titrated on microtiter plates coated with the immunogen (peptide 643-683, SEQ .ID. NO.6) as well as on

-19-

plates coated with the purified recombinant HCV E2/NS1 protein PHCV80 (a.a. 365-731, SEQ ID NO.7) by the EIA protocol described in Example 1. The detail description of cloning and expression of pHCV80 is described in Example 6. EIA reactivity of monoclonal antibodies of this invention to the 5 immunogen as well as the recombinant HCV E2/NS1 protein is described in Table 1.

#### Western Blot Analysis

Approximately 300 µg of the HCV protein PHCV-80 (a.a. 365-731, 10 SEQ. ID. NO. 7) were treated with SDS and 2-mercaptoethanol at 95°C, and electrophoresed in a 12% polyacrylamide-SDS gel (Laemmli et al., *Nature* 227:680-685 (1970). Proteins were transferred overnight from the gel to nitrocellulose by electrophoresis at 100 mamp, or transferred in 1-2 hours at 1.0 amp, in a standard transfer buffer which comprised 25 mM Tris 15 [(Hydroxymethyl) Aminomethane] 192 mM glycine, and 2.0% methanol, pH 8.3. (Towbin et al., *Proc. Natl. Acad. Sci.* 73:4350-4354 [1979]). After transferring the proteins and blocking the nitrocellulose with 5% dry milk in PBS, the nitrocellulose was cut into strips (each strip containing approximately 5 µg of the protein which then were used to determine the 20 presence of anti-HCV antibody in test sera (or other samples). Reaction mixtures consisted of a nitrocellulose strip incubated with an appropriate amount of test sample in 2.0 ml of buffer (20 mM Tris, 1 mM EDTA, 0.2 M NaCl, 0.3% Triton X-100® and 2 mg/ml bovine serum albumin (BSA), pH 7.5, 5% E. coli lysate and 3% CKS lysate overnight at 4°C. The strips were 25 washed with buffered detergent (10 mM phosphate buffered saline (PBS) pH 7.5, containing 0.1% SDS and 0.5% Triton X-100®), followed by addition of goat anti-mouse IgG antibody conjugated to HRPO. The strips were incubated for one to two hours at room temperature, followed by washing with buffered detergent. Finally, antibody bound to the protein was visualized by 30 addition of freshly prepared HRP color reagent (Bio-Rad Laboratories, Richmond CA) (120 mg dissolved in 40 ml ice-cold methanol, then diluted into 200 ml Tris buffered saline [TBS] pH 7.8, containing 120 µl of 30% hydrogen peroxide. Fig 2 illustrates the specific reactivity of the monoclonals of this invention to the HCV E2/NS1 protein.

35

#### Competition with Immune Human Sera

In order to establish whether each of the monoclonal antibodies recognized an epitope that is immunolgic in humans, a competition assay was performed as follows. Each of the monoclonal antibodies was tested in

-20-

an assay where the monoclonal antibody competed with a human sera seropositive for antibody to E2/NS1 (SEQ. ID. NO. 1) for the binding to the antigen. Briefly, a human serum from an individual infected with NANBH and strongly seropositive for antibodies to E2/NS1 protein of HCV was included in  
5 the reaction mixture with each of the monoclonal antibodies at a final concentration of 10%. Microtiter EIA was carried out as described in Example 1. A greater than 50% inhibition in the binding of the monoclonal antibody to the respective protein by the immune human sera was considered as competitive (data presented in Table 1). Monoclonal antibodies H13C113  
10 and H23C163 were not significantly competed by sera from individuals seropositive for antibodies to HCV E2/NS1.

Isotype

The isotypes of each of the monoclonal antibodies was determined by  
15 using an isotyping kit (Amersham, Arlington Heights, IL) and following the instructions included with it. Briefly, the tissue culture supernatant of each monoclonal antibody and appropriate controls were reacted at a 1:5 dilution with strips coated with specific anti-isotype antibody, provided in the kit described above. Assay protocol was followed exactly according to the  
20 manufacturer's instructions. The isotype of each monoclonal antibody of the invention is provided in TABLE 1.

EXAMPLE 6

Epitope Mapping

25 Monoclonal antibodies generated against the synthetic peptide (SEQ.ID.NO.6) were mapped to the specific region of the HCV E2/NS1 protein by (a) Western blot reactivity of each of the monoclonal antibodies with subfragments of the HCV E2/NS1 protein and (b) reactivity with several synthetic peptides selected for respective protein sequences, by microtiter  
30 EIA using the procedure described in Example 1.

Reactivity of Monoclonals to Various Subfragments of Recombinant HCV NS1 proteins

Briefly, several individual oligonucleotides representing a.a. 365-731  
35 of HCV genome were ligated and cloned as three separate EcoRI-BAMHI subfragments into the CKS fusion vector pJ0200. These three subfragments were designated as pHCV80 (a.a. 365-731) ( SEQ.ID.NO.7), pHCV77 (a.a. 365-579) (SEQ. ID. NO. 8), and pHCV65 (a.a. 565-731) (SEQ. ID. NO. 9), as illustrated in FIG. 2 . The detailed methods for cloning and expression of the

-21-

CKS-fusion proteins are as disclosed in U.S. Patent Application Serial No. 07/610,180 and 07/572,822, which enjoy common ownership and are incorporated herein by reference. Cell lysates of these clones were used as antigens on Western blot analysis using the protocol described in Example 5

5 for preliminary epitope mapping of anti-NS1 monoclonal antibodies. Fig 2 shows the binding of monoclonal antibodies H13C113 and H23C163 to recombinant HCV E2/NS1 protein subfragments, wherein lane 1 (normal human sera), lane 2( HCV immune human sera), and lane 3( normal mouse sera) were included as controls. Lane 4 contains hybrid supernatant from

10 which H13C113 was cloned, lane 6 contains monoclonal antibody H13C113, lane 5 contains a sister clone of monoclonal antibody H13C113 (H13C44), lane 10 contains monoclonal antibody H23C163, while lanes 8 and 9 contain sister clones of monoclonal antibody H23C163 (H23C41 and H23C41 respectively). Data for epitope mapping with these subfragments are

15 illustrated in FIG. 2. Monoclonal antibodies H13C113 and H23C163 showed reactivity with pHCV 80 (SEQ. ID. NO. 7) and pHCV 65 (SEQ. ID. NO. 9) which indicated the reactivity with HCV a.a. 565-731 (SEQ. ID. NO. 9).

#### Reactivity with Synthetic Peptides

20 Several amino acid sequences were selected from different regions of HCV protein NS1 based on the PEPSCAN analysis as described in Example 1. A list of the peptides used for the epitope mapping of these monoclonal antibodies is listed below in TABLE 2.

-22-

TABLE 2

## Epitope Mapping with Synthetic Peptides

5	REGION OF HCV GENOME PEPTIDE	MONOCLONAL TESTED	PEPTIDE a.a.	REACTIVITY OF EACH WITH
10	NS1	H13C113	sp 643-663	sp 643-663
		H23C163	sp 643-683	sp. 643-683
			sp. 666-683	

Each of these peptides were assembled on a resin support by a stepwise solid phase synthesis, starting with the carboxy terminal residue. A procedures was employed similar to that described in E. Gross and T.

15 Heinehofer, eds., Barany and Merrifield, The Peptides 2:1284, Academic Press, New York, New York (1980), using a reaction vessel of an Applied Biosystems Synthesizer Model 430A. After cleavage of the peptide from the resin, the peptide was washed with diethyl ether and extracted in 40% acetic acid solution. Crude peptide obtained after lyophilization of the aqueous  
20 solution was employed as the antigen target for epitope mapping experiments. Briefly, each of the peptides tested was coated on microtiter wells at a concentration of 10 µg/ml in bicarbonate buffer at pH 9.5. EIA was performed in the manner described in Example 1. Monoclonal antibody showing reactivity four times the negative control was considered positive.

25 In addition, monoclonal antibodies to HCV NS1 were also mapped with PEPSCAN analysis as described in Example 1. An EIA was performed with each of the monoclonal antibodies to HCV NS1 by the procedures similar to one outlined in Example 1 using the tissue culture supernatants of  
30 monoclonal antibodies as the primary antibody and goat anti-mouse HRPO as the secondary antibody with overlapping hexamer peptides encompassing a.a. 600-720 (SEQ. ID. NO. 1) of the HCV genome. Data are illustrated in Fig.. 3 and Fig 4. Monoclonal antibody H13C113 and H23C163 specifically reacted with peptide sequence GDRCDLE (a.a. 649-655) (SEQ. ID. NO. 10)  
35 of the HCV genome.

-23-

### EXAMPLE 7

#### EIA for the Detection of HCV Proteins in Biological Samples

##### Preparation of Rabbit Polyclonal Antibodies Against HCV E2/NS1 Region

Young rabbits (3-4 months old and weighing approximately 2-3 kg)

5 (available from Hazelton Labs, Denver PA) are immunized with 100-150 µg of highly purified HCV E2/NS1 synthetic peptide or the E2/NS1 recombinant proteins cloned and expressed in either eukaryotic or prokaryotic systems as described in Example 1 in Freund's complete adjuvant by intra-muscular (i.m.) injection at four different sites. Subsequently, two immunizations are  
10 carried out at two week intervals in similar fashion in Freund's incomplete adjuvant. Immune response of the rabbits is monitored by EIA and Western blot analysis. Rabbits are bled when acceptable immune response to the protein is achieved. IgG from the immune rabbit sera is purified by Protein-A sepharose affinity chromatography, by methods known to those in the art.

15

##### Coating of Solid Phase

Rabbit IgG is prepared as herein described and then is coated on polystyrene beads as the solid support for capture of E2/NS1 antigens in test samples. The polystyrene beads are washed with distilled water and  
20 incubated at 40°C for two hours with 5-10 µg/ml of purified HCV E2/NS1 synthetic peptide rabbit IgG in a buffer solution (0.1 M Tris, 0.5 M NaCl, 0.0022% Triton X-100®, pH 8.5). The beads are washed once with PBS and then soaked in 0.1% Triton X-100® in PBS for approximately one hour at 40°C. After washing twice with PBS, the beads are overcoated with 3%  
25 bovine serum albumin (BSA) in PBS for approximately one hour at 40°C. Finally, the beads are overcoated with 5% sucrose solution in PVS and dried under nitrogen. Anti-HCV human polyclonal IgG, purified from sera of individuals seropositive for HCV antibodies to E2/NS1 also is coated in similar fashion.

30

##### EIA

Monoclonal antibodies specific for HCV E2/NS1 are screened for use as the probe for detection of HCV proteins in a test sample by EIA. Briefly, each of the monoclonal antibodies is incubated with the E2/NS1 antigen in  
35 the presence of polystyrene beads coated with anti-HCV rabbit polyclonal IgG. The protocol for EIA is similar to that described hereinbelow.

200 µl of test specimen suspected of containing antigen to HCV E2/NS1 protein is incubated in a reaction tray with 50 µl of monoclonal

-24-

antibody of the invention (at a final protein concentration of about 5-10  $\mu$ g/ml diluted in a buffer containing 20 mM Tris, 0.1 mM NaCl, 1mM EDTA, 3.0% BSA, 0.3% Tween-20® and 10% FBS at pH 7.5), and a bead coated with HCV rabbit IgG (prepared as described hereinabove). Overnight incubation

5 at ambient room temperature is performed, and then the beads are washed with distilled water and 200  $\mu$ l of appropriately dilution Horseradish Peroxidase labeled goat anti-mouse IgG (H & L) (Jackson Immunoresearch, West Grove, PA) is added. Incubation with the labeled probe is carried out at about 40°C for approximately two hours. Beads are washed and transferred

10 to reaction tubes containing 300  $\mu$ l of O-phenylenediamine (OPD) color reagent. The reaction is carried out at ambient room temperature in the dark for about 30 minutes, and then it is stopped by the addition of 1 ml of 1N H<sub>2</sub>SO<sub>4</sub>. Absorbance is recorded at 492/600 nm. A negative control previously screened and confirmed to be negative for NANBH infection is

15 included in the experiment. The positive control consists of a solution of synthetic peptide to E2/NS1 in the buffer solution described hereinabove. Triplicates of both positive and negative control are included with each set of experiments.

20 In order to determine the efficiency of the antigen capture assay for the detection of HCV E2/NS1 in a sample, various concentrations of recombinant HCV E2/NS1 synthetic peptide, ranging from 100 ng peptide/ml to 100 pg peptide/ml are diluted in the buffer mentioned hereinabove. The EIA procedure described above is performed with each of the diluted panel

25 members. For the purposes of comparison, each of the panel members is tested with (a) anti-HCV rabbit polyclonal antibody on the solid phase and (b) anti-HCV human polyclonal antibody on the solid phase. The efficiency of the assay then is determined by evaluating data obtained.

30 The hybridomas which produce the monoclonal antibodies of the invention are identified as hybridoma H13C113 producing monoclonal antibody H13C113, and hybridoma H23C163 producing monoclonal antibody H23C163. Hybridomas H13C113 and H23C163 were deposited at the American Type Culture Collection (ATCC), 12301 Parklawn Drive,

35 Rockville, Maryland 20852 as of August 20, 1991, and have been accorded the following deposit numbers: hybridoma H13C113 was accorded ATCC deposit number HB 10857, and hybridoma H23C163 was accorded ATCC deposit number HB 10856.

-25-

Thus, the novel monoclonal antibodies of the invention can be used in a variety of ways. These monoclonal antibodies can be used for immunoprecipitation of amplified product and detection of HCV nucleic acid microparticles or carrier coated with anti-HCV monoclonal antibody used to capture virus or viral protein associated with HCV RNA. Then detection methodology for RNA may be used. An example of this type of assay is taught in pending U. S. patent application Serial No. 07/568,663, entitled A METHOD FOR AMPLIFYING AND DETECTING A TARGET NUCLEIC ACID SEQUENCE, which enjoys common ownership and is incorporated herein by reference.

These monoclonal antibodies also can be used for localization of HCV antigens within the cell using HCV monoclonal antibody tagged directly (fluorescence, colloidal gold, etc.) or using secondary tagged anti-mouse antibody. Histopathology of disease may be tracked. Further, the detection of native or recombinant HCV antigens in sera, tissue, cells, culture media, or body fluid using individual monoclonal antibodies in a sandwich configuration or a cocktail of monoclonal antibodies on the solid phase and in the detection system.

One step antigen assays using monoclonal antibodies against non overlapping epitopes may also be performed. Some monoclonal antibodies may recognize antigenic epitopes not recognized by the infected individual and therefore may be possible to recognize serum Ag both free and bound with human antibody. Furthermore, "cryptic" or hidden antigens or antigenic determinants may be uncovered by treatment of specimen with detergent or reducing agent or both. For example, CORE antigen may exist in a capsid form covered by the virus envelope. Stripping the envelope with detergent should expose CORE antigen. Monoclonal antibodies may also offer pragmatic advantages over high titer polyclonal antibody in giving greater sensitivity in assay or allowing shorter incubation times.

Further, antibody immunoassays, one or two step competitive assays, were developed in which anti-HCV competed with labeled anti-HCV monoclonal antibody for binding to a limited number of antigenic sites. A more sensitive competitive assay may be developed in which human anti-HCV binds to HCV Ag in solution blocking or inhibiting the HCV Ag binding in HCV Ag sandwich assay. Competitive assays using monoclonal antibodies allow a more precise mapping of human antibody epitopes and may be

-26-

useful for determining virus neutralizing antibody epitopes. Some monoclonal antibodies may have virus neutralizing activity. Finally, monoclonal antibodies should be useful in immunoaffinity purification of native viral and recombinant HCV antigens and proteins.

5

Other variations of applications of the use of these unique monoclonal antibodies provided herein include the detection of HCV in immune complexes, or latent and/or cryptic antigens, and/or associated with viral nucleic acid for detection of the nucleic acid by PCR, LCR, or by direct 10 hybridization. Still other variations and modifications of the specific embodiments of the invention as set forth herein will be apparent to those skilled in the art. Accordingly, the invention is intended to be limited only in accordance with the appended claims.

15

TABLE 1

MONOCLONAL ANTIBODIES TO HCV NS1 PROTEIN

IMMUNOGEN	MAB ID	ISOTYPE	COMP WITH IMMUNE H.U.SERA	WESTERN BLOT			TITER	EPITOPE
				pHCV-65 <sup>a</sup>	pHCV-80 <sup>b</sup>	643-683 <sup>b</sup>		
sp 643-683	H13C113	IgG3,k	-	+	+	+	10ng/ml 80ng/ml	649-655 <sup>c</sup>
	H23C163	IgG2b,k	-	+	+	+	80ng/ml 1ug/ml	649-655

- a. pHCV-65 a.a. 565-731
- b. pHCV-80 a.a. 365-731
- c. a.a. sequence =Gly-Glu-Arg-Cys-Asp-Leu-Glu

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: MEHTA, SMRITI U.  
JOHNSON, JILL E.  
DAILEY, STEPHEN H.  
DESAI, SURESH M.  
DEVARE, SUSHIL G.

(ii) TITLE OF INVENTION: MONOCLONAL ANTIBODIES TO PUTATIVE HCV  
E2/NS1 PROTEINS AND METHODS FOR USING SAME

(iii) NUMBER OF SEQUENCES: 10

## (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: ABBOTT LABORATORIES D-377/AP6D  
(B) STREET: ONE ABBOTT PARK ROAD  
(C) CITY: ABBOTT PARK  
(D) STATE: ILLINOIS  
(E) COUNTRY: USA  
(F) ZIP: 60064-3500

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/748,292  
(B) FILING DATE: 21-AUG-1991  
(C) CLASSIFICATION:

## (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/456,162  
(B) FILING DATE: 22-DEC-1989

## (viii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 07/610,180  
(B) FILING DATE: 07-NOV-1990

## (ix) ATTORNEY/AGENT INFORMATION:

(A) NAME: POREMBSKI, PRISCILLA E.  
(B) REGISTRATION NUMBER: 33,207  
(C) REFERENCE/DOCKET NUMBER: 4767.US.P2

## (x) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 708-937-6365  
(B) TELEFAX: 708-937-9556

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 121 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gly	Pro	Trp	Ile	Thr	Pro	Arg	Cys	Leu	Val	Asp	Tyr	Pro	Tyr	Arg	Leu
1															15
Trp	His	Tyr	Pro	Cys	Thr	Ile	Asn	Tyr	Thr	Ile	Phe	Lys	Ile	Arg	Met
															30
Tyr	Val	Gly	Gly	Val	Glu	His	Arg	Leu	Glu	Ala	Ala	Cys	Asn	Trp	Thr
															45
Arg	Gly	Glu	Arg	Cys	Asp	Leu	Glu	Asp	Arg	Asp	Arg	Ser	Glu	Leu	Ser
															60
Pro	Leu	Leu	Leu	Thr	Thr	Thr	Gln	Trp	Gln	Val	Leu	Pro	Cys	Ser	Phe
															80
Thr	Thr	Leu	Pro	Ala	Leu	Ser	Thr	Gly	Leu	Ile	His	Leu	His	Gln	Asn
															95
Ile	Val	Asp	Val	Gln	Tyr	Leu	Tyr	Gly	Val	Gly	Ser	Ser	Ile	Ala	Ser
															110
Trp	Ala	Ile	Lys	Trp	Glu	Tyr	Val	Val							
															120

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Cys Leu Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Ile  
1 5 10 15

Asn Tyr Thr Ile Phe  
20

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp Arg Asp  
1 5 10 15

Arg Ser Glu Leu Ser  
20

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Leu Leu Thr Thr Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr  
1 5 10 15

Leu Pro

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu Ser  
1 5 10 15

Thr Gly Leu Ile His  
20

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 41 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp Arg Asp  
1 5 10 15

Arg Ser Glu Leu Ser Pro Leu Leu Thr Thr Thr Gln Trp Gln Val  
20 25 30

Leu Pro Cys Ser Phe Thr Thr Leu Pro  
35 40

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 621 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ser Phe Val Val Ile Ile Pro Ala Arg Tyr Ala Ser Thr Arg Leu

1 5 10 15

Pro Gly Lys Pro Leu Val Asp Ile Asn Gly Lys Pro Met Ile Val His  
20 25 30

Val Leu Glu Arg Ala Arg Glu Ser Gly Ala Glu Arg Ile Ile Val Ala  
35 40 45

Thr Asp His Glu Asp Val Ala Arg Ala Val Glu Ala Ala Gly Gly Glu  
50 55 60

Val Cys Met Thr Arg Ala Asp His Gln Ser Gly Thr Glu Arg Leu Ala  
65 70 75 80

Glu Val Val Glu Lys Cys Ala Phe Ser Asp Asp Thr Val Ile Val Asn  
85 90 95

Val Gln Gly Asp Glu Pro Met Ile Pro Ala Thr Ile Ile Arg Gln Val  
100 105 110

Ala Asp Asn Leu Ala Gln Arg Gln Val Gly Met Thr Thr Leu Ala Val  
115 120 125

Pro Ile His Asn Ala Glu Glu Ala Phe Asn Pro Asn Ala Val Lys Val  
130 135 140

Val Leu Asp Ala Glu Gly Tyr Ala Leu Tyr Phe Ser Arg Ala Thr Ile  
145 150 155 160

Pro Trp Asp Arg Asp Arg Phe Ala Glu Gly Leu Glu Thr Val Gly Asp  
165 170 175

Asn Phe Leu Arg His Leu Gly Ile Tyr Gly Tyr Arg Ala Gly Phe Ile  
180 185 190

Arg Arg Tyr Val Asn Trp Gln Pro Ser Pro Leu Glu His Ile Glu Met  
195 200 205

Leu Glu Gln Leu Arg Val Leu Trp Tyr Gly Glu Lys Ile His Val Ala  
210 215 220

Val Ala Gln Glu Val Pro Gly Thr Gly Val Asp Thr Pro Glu Asp Leu  
225 230 235 240

Asp Pro Ser Thr Asn Ser Thr Met Val Gly Asn Trp Ala Lys Val Leu  
245 250 255

Val Val Leu Leu Leu Phe Ala Gly Val Asp Ala Glu Thr His Val Thr  
260 265 270

Gly Gly Ser Ala Gly His Thr Val Ser Gly Phe Val Ser Leu Leu Ala

275                    280                    285

Pro Gly Ala Lys Gln Asn Val Gln Leu Ile Asn Thr Asn Gly Ser Trp  
290                    295                    300

His Leu Asn Ser Thr Ala Leu Asn Cys Asn Asp Ser Leu Asn Thr Gly  
305                    310                    315                    320

Trp Leu Ala Gly Leu Phe Tyr His His Lys Phe Asn Ser Ser Gly Cys  
325                    330                    335

Pro Glu Arg Leu Ala Ser Cys Arg Pro Leu Thr Asp Phe Asp Gln Gly  
340                    345                    350

Trp Gly Gln Ile Ser Tyr Ala Asn Gly Ser Gly Pro Asp Gln Arg Pro  
355                    360                    365

Tyr Cys Trp His Tyr Pro Pro Lys Pro Cys Gly Ile Val Pro Ala Lys  
370                    375                    380

Ser Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val Val  
385                    390                    395                    400

Gly Thr Thr Asp Arg Ser Gly Ala Pro Thr Tyr Ser Trp Gly Glu Asn  
405                    410                    415

Asp Thr Asp Val Phe Val Leu Asn Asn Thr Arg Pro Pro Leu Gly Asn  
420                    425                    430

Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly Phe Thr Lys Val Cys  
435                    440                    445

Gly Ala Pro Pro Cys Val Ile Gly Pro Pro Cys Val Ile Gly Gly Ala  
450                    455                    460

Gly Asn Asn Thr Leu His Cys Pro Thr Asp Cys Phe Arg Lys His Pro  
465                    470                    475                    480

Asp Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro Arg  
485                    490                    495

Cys Leu Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr Ile  
500                    505                    510

Asn Tyr Thr Ile Phe Lys Ile Arg Met Tyr Val Gly Gly Val Glu His  
515                    520                    525

Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu  
530                    535                    540

Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Thr Thr Thr

34

545 550 555 560

Gin Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu Ser  
565 570 575Thr Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val Gin Tyr Leu  
580 585 590Tyr Gly Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp Glu Tyr  
595 600 605Val Val Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Xaa  
610 615 620

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 414 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ser Phe Val Val Ile Ile Pro Ala Arg Tyr Ala Ser Thr Arg Leu  
1 5 10 15Pro Gly Lys Pro Leu Val Asp Ile Asn Gly Lys Pro Met Ile Val His  
20 25 30Val Leu Glu Arg Ala Arg Glu Ser Gly Ala Glu Arg Ile Ile Val Ala  
35 40 45Thr Asp His Glu Asp Val Ala Arg Ala Val Glu Ala Ala Gly Gly Glu  
50 55 60Val Cys Met Thr Arg Ala Asp His Gln Ser Gly Thr Glu Arg Leu Ala  
65 70 75 80Glu Val Val Glu Lys Cys Ala Phe Ser Asp Asp Thr Val Ile Val Asn  
85 90 95Val Gln Gly Asp Glu Pro Met Ile Pro Ala Thr Ile Ile Arg Gin Val  
100 105 110Ala Asp Asn Leu Ala Gln Arg Gin Val Gly Met Thr Thr Leu Ala Val  
115 120 125

Pro Ile His Asn Ala Glu Glu Ala Phe Asn Pro Asn Ala Val Lys Val  
130 135 140

Val Leu Asp Ala Glu Gly Tyr Ala Leu Tyr Phe Ser Arg Ala Thr Ile  
145 150 155 160

Pro Trp Asp Arg Asp Arg Phe Ala Glu Gly Leu Glu Thr Val Gly Asp  
165 170 175

Asn Phe Leu Arg His Leu Gly Ile Tyr Gly Tyr Arg Ala Gly Phe Ile  
180 185 190

Arg Arg Tyr Val Asn Trp Gln Pro Ser Pro Leu Glu His Ile Glu Met  
195 200 205

Leu Glu Gln Leu Arg Val Leu Trp Tyr Gly Glu Lys Ile His Val Ala  
210 215 220

Val Ala Gln Glu Val Pro Gly Thr Gly Val Asp Thr Pro Glu Asp Leu  
225 230 235 240

Asp Pro Ser Thr Asn Ser Met Gly Ala Pro Pro Cys Val Ile Gly Gly  
245 250 255

Ala Gly Asn Asn Thr Leu His Cys Pro Thr Asp Cys Phe Arg Lys His  
260 265 270

Pro Asp Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro Trp Ile Thr Pro  
275 280 285

Arg Cys Leu Val Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys Thr  
290 295 300

Ile Asn Tyr Thr Ile Phe Lys Ile Arg Met Tyr Val Gly Gly Val Glu  
305 310 315 320

His Arg Leu Glu Ala Ala Cys Asn Trp Thr Arg Gly Glu Arg Cys Asp  
325 330 335

Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu Leu Leu Thr Thr  
340 345 350

Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr Leu Pro Ala Leu  
355 360 365

Ser Thr Gly Leu Ile His Leu His Gln Asn Ile Val Asp Val Gln Tyr  
370 375 380

Leu Tyr Gly Val Gly Ser Ser Ile Ala Ser Trp Ala Ile Lys Trp Glu  
385 390 395 400

Tyr Val Val Leu Leu Phe Leu Leu Ala Asp Ala Arg Val  
405 410

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 463 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Ser Phe Val Val Ile Ile Pro Ala Arg Tyr Ala Ser Thr Arg Leu  
1 5 10 15

Pro Gly Lys Pro Leu Val Asp Ile Asn Gly Lys Pro Met Ile Val His  
20 25 30

Val Leu Glu Arg Ala Arg Glu Ser Gly Ala Glu Arg Ile Ile Val Ala  
35 40 45

Thr Asp His Glu Asp Val Ala Arg Ala Val Glu Ala Ala Gly Gly Glu  
50 55 60

Val Cys Met Thr Arg Ala Asp His Gln Ser Gly Thr Glu Arg Leu Ala  
65 70 75 80

Glu Val Val Glu Lys Cys Ala Phe Ser Asp Asp Thr Val Ile Val Asn  
85 90 95

Val Gln Gly Asp Glu Pro Met Ile Pro Ala Thr Ile Ile Arg Gln Val  
100 105 110

Ala Asp Asn Leu Ala Gln Arg Gln Val Gly Met Thr Thr Leu Ala Val  
115 120 125

Pro Ile His Asn Ala Glu Glu Ala Phe Asn Pro Asn Ala Val Lys Val  
130 135 140

Val Leu Asp Ala Glu Gly Tyr Ala Leu Tyr Phe Ser Arg Ala Thr Ile  
145 150 155 160

Pro Trp Asp Arg Asp Arg Phe Ala Glu Gly Leu Glu Thr Val Gly Asp  
165 170 175

Asn Phe Leu Arg His Leu Gly Ile Tyr Gly Tyr Arg Ala Gly Phe Ile

180 185 190

Arg Arg Tyr Val Asn Trp Gln Pro Ser Pro Leu Glu His Ile Glu Met  
195 200 205

Leu Glu Gln Leu Arg Val Leu Trp Tyr Gly Glu Lys Ile His Val Ala  
210 215 220

Val Ala Gln Glu Val Pro Gly Thr Gly Val Asp Thr Pro Glu Asp Leu  
225 230 235 240

Asp Pro Ser Thr Asn Ser Thr Met Val Gly Asn Trp Ala Lys Val Leu  
245 250 255

Val Val Leu Leu Leu Phe Ala Gly Val Asp Ala Glu Thr His Val Thr  
260 265 270

Gly Gly Ser Ala Gly His Thr Val Ser Gly Phe Val Ser Leu Leu Ala  
275 280 285

Pro Gly Ala Lys Gln Asn Val Gln Leu Ile Asn Thr Asn Gly Ser Trp  
290 295 300

His Leu Asn Ser Thr Ala Leu Asn Cys Asn Asp Ser Leu Asn Thr Gly  
305 310 315 320

Trp Leu Ala Gly Leu Phe Tyr His His Lys Phe Asn Ser Ser Gly Cys  
325 330 335

Pro Glu Arg Leu Ala Ser Cys Arg Pro Leu Thr Asp Phe Asp Gln Gly  
340 345 350

Trp Gly Gln Ile Ser Tyr Ala Asn Gly Ser Gly Pro Asp Gln Arg Pro  
355 360 365

Tyr Cys Trp His Tyr Pro Pro Lys Pro Cys Gly Ile Val Pro Ala Lys  
370 375 380

Ser Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val Val  
385 390 395 400

Gly Thr Thr Asp Arg Ser Gly Ala Pro Thr Tyr Ser Trp Gly Glu Asn  
405 410 415

Asp Thr Asp Val Phe Val Leu Asn Asn Thr Arg Pro Pro Leu Gly Asn  
420 425 430

Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly Phe Thr Lys Val Cys  
435 440 445

Gly Ala Pro Pro Cys Val Ile Gly Gly Ala Gly Asn Asn Thr Leu

450                    455                    460

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gly Asp Arg Cys Asp Leu Glu  
1                    5

## WE CLAIM:

1. A monoclonal antibody which specifically binds to HCV E2/NS1 antigen.  
5
2. The monoclonal antibody of claim 1 wherein said monoclonal antibody is the monoclonal antibody secreted by hybridoma cell line ATCC HB 10857.
- 10 3. The monoclonal antibody of claim 1 wherein said monoclonal antibody is the monoclonal antibody secreted by hybridoma cell line ATCC HB 10856.
- 15 4. A monoclonal antibody secreted by A.T.C.C. deposit No. HB 10857.
5. A monoclonal antibody secreted by A.T.C.C. deposit No. HB 10856.
- 20 6. A hybridoma cell line which secretes a monoclonal antibody which specifically binds to HCV E2/NS1 antigen.
7. The hybridoma cell line of claim 6 wherein said hybridoma cell line is A.T.C.C. deposit No. HB 10857.  
25
8. The hybridoma cell line of claim 6 wherein said hybridoma cell line is A.T.C.C. deposit No. HB 10856.
9. A hybridoma cell line A.T.C.C. deposit No. HB 10857.
- 30 10. A hybridoma cell line A.T.C.C. deposit No. HB 10856.
11. A method for determining the presence of HCV in a test sample which may contain HCV, comprising:  
35
- a. contacting the test sample with at least one anti-HCV E2/NS1 antibody attached to a solid phase which antibody specifically binds to HCV E2/NS1 antigen, to form a mixture;

- b. incubating said mixture for a time and under conditions sufficient to form antigen/antibody complexes;
  - c. contacting said complexes with an indicator reagent comprising a signal generating compound capable of generating a measurable detectable signal attached to an anti-HCV E2/NS1 antibody, to form a second mixture;
  - d. incubating said second mixture for a time and under conditions sufficient to form antibody/antigen/antibody complexes; and
- 10 e. determining the presence of HCV in the test sample by detecting the measurable signal generated, wherein the amount of HCV present in the test sample is proportional to said measurable signal.
- 15 12. The method of claim 11 wherein the signal generating compound is selected from the group consisting of a luminescent compound, a chemiluminescent compound, an enzyme and a radioactive element.
- 20 13. The method of claim 11 wherein the anti-HCV antibody attached to the solid phase is a polyclonal antibody.
- 25 14. The method of claim 11 wherein said anti-HCV E2/NS1 antibody attached to the solid phase is a monoclonal antibody.
- 30 15. The method of claim 14 wherein said monoclonal antibody is the monoclonal antibody secreted by the hybridoma A.T.C.C. deposit No. HB 10857.
- 35 16. The method of claim 14 wherein said monoclonal antibody is the monoclonal antibody secreted by the hybridoma A.T.C.C. deposit No. HB 10856.
17. The method of claim 11 wherein said indicator reagent comprises a signal generating compound attached to a polyclonal antibody.

18. The method of claim 11 wherein said indicator reagent comprises a signal generating compound attached to a monoclonal antibody.

5 19. The method of claim 18 wherein said monoclonal antibody is the monoclonal antibody secreted by the hybridoma cell line A.T.C.C. deposit No. HB 10857.

10 20. The method of claim 18 wherein said monoclonal antibody is the monoclonal antibody secreted by the hybridoma cell line A.T.C.C. deposit No. HB 10856.

21. A method for determining the presence and amount of HCV which may be present in a test sample, comprising:

15 a. contacting a test sample with a polyclonal or monoclonal anti-HCV E2/NS1 antibody attached to a solid phase and an indicator reagent comprising a monoclonal or polyclonal antibody which specifically binds to HCV E2/NS1 attached to a signal generating compound, to form a mixture;

20 b. incubating said mixture for a time and under conditions sufficient to form antibody/antigen/antibody complexes;

25 c. determining the presence of HCV present in the test sample by detecting the measurable signal as an indication of the presence of HCV in the test sample, wherein the amount of HCV present in the test sample is proportional to the measurable signal generated.

22. The method of claim 21 wherein said monoclonal antibody is the monoclonal antibody secreted by the hybridoma cell line A.T.C.C. deposit No. HB 10857.

23. The method of claim 21 wherein said monoclonal antibody is the monoclonal antibody secreted by the hybridoma cell line A.T.C.C. deposit No. HB 10856.

35

24. A competitive assay method for determining the presence and amount of HCV antibody which may be present in a test sample, comprising:

- a. contacting a test sample suspected of containing HCV antibodies with a solid phase coated with HCV E2/NS1 proteins and an indicator reagent comprising a signal generating compound and a monoclonal antibody which
- 5 specifically binds to HCV E2/NS1 proteins, for a time and under conditions sufficient to form antigen/antibody complexes of the test sample and solid phase and/or indicator reagent and solid phase;
- b. determining the presence of HCV antibody present in the test sample by detecting the reduction in binding of
- 10 the indicator reagent to the solid phase as compared to the signal generated from a negative test sample to indicate the presence of HCV antibody in the test sample

25. The method of claim 24 wherein said monoclonal

15 antibody is the monoclonal antibody secreted by the hybridoma cell line A.T.C.C. deposit No. HB 10857.

26. The method of claim 24 wherein said monoclonal antibody is the monoclonal antibody secreted by the hybridoma cell

20 line A.T.C.C. deposit No. HB 10856.

27. An assay kit for determining the presence of HCV antigen or antibody in a test sample comprising:  
a container containing at least one monoclonal

25 antibody which specifically binds to HCV E2/NS1 antigen.

28. The assay kit of claim 27 wherein said monoclonal antibody is the monoclonal antibody secreted by the cell line A.T.C.C. deposit No. HB 10857.

30 29. The assay kit of claim 27 wherein said monoclonal antibody is the monoclonal antibody secreted by the cell line A.T.C.C. deposit No. HB 10856.

1/4

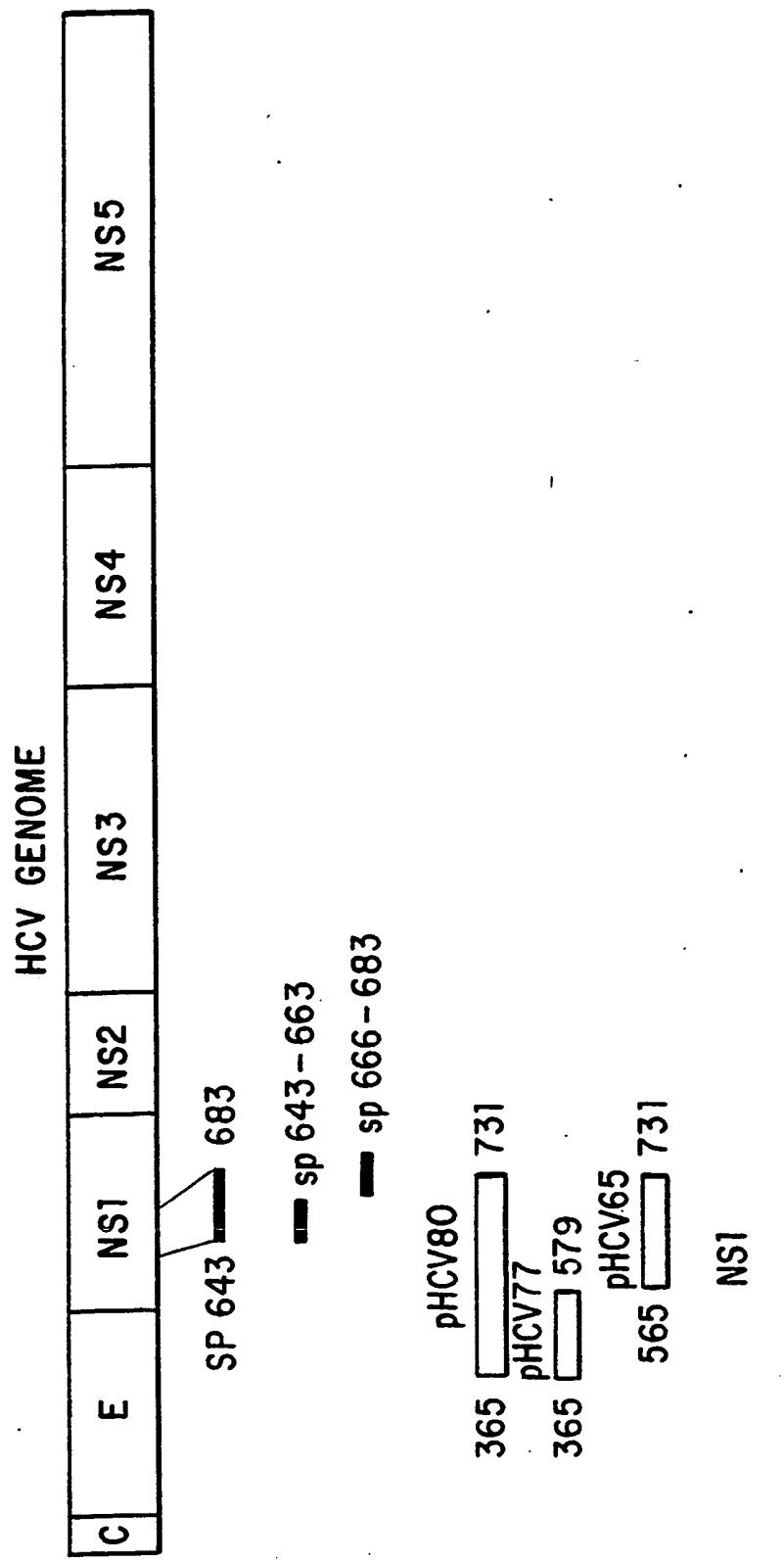


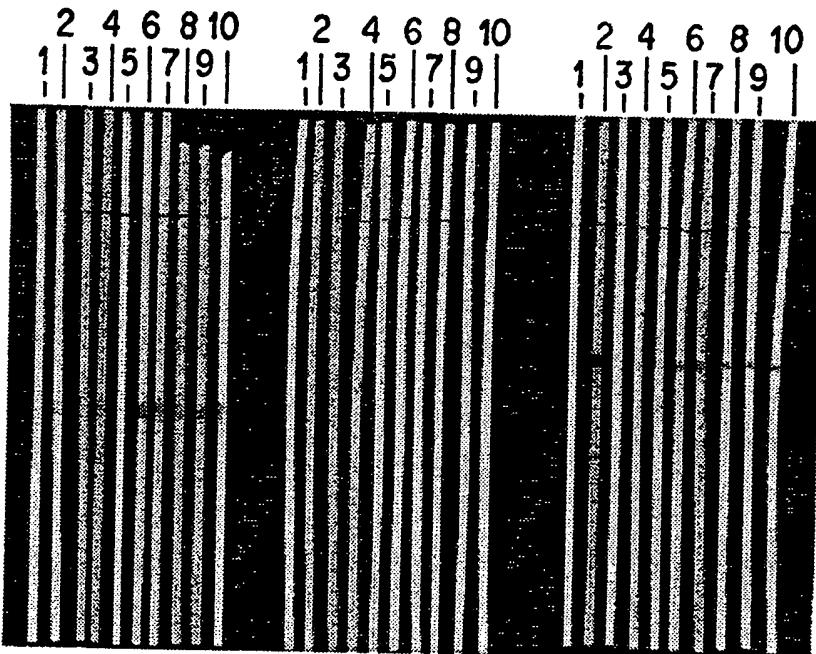
FIG. 1

2/4

pHCV-65  
aa. 565-731

pHCV-77  
aa. 365-579

pHCV80  
aa. 365-841



1 - NHS      4 - H13      7 - H23      10 - H23C163  
2 - IHS      5 - H13C44      8 - H23C41  
3 - NM      6 - H13C113      9 - H23C115

FIG. 2

3/4

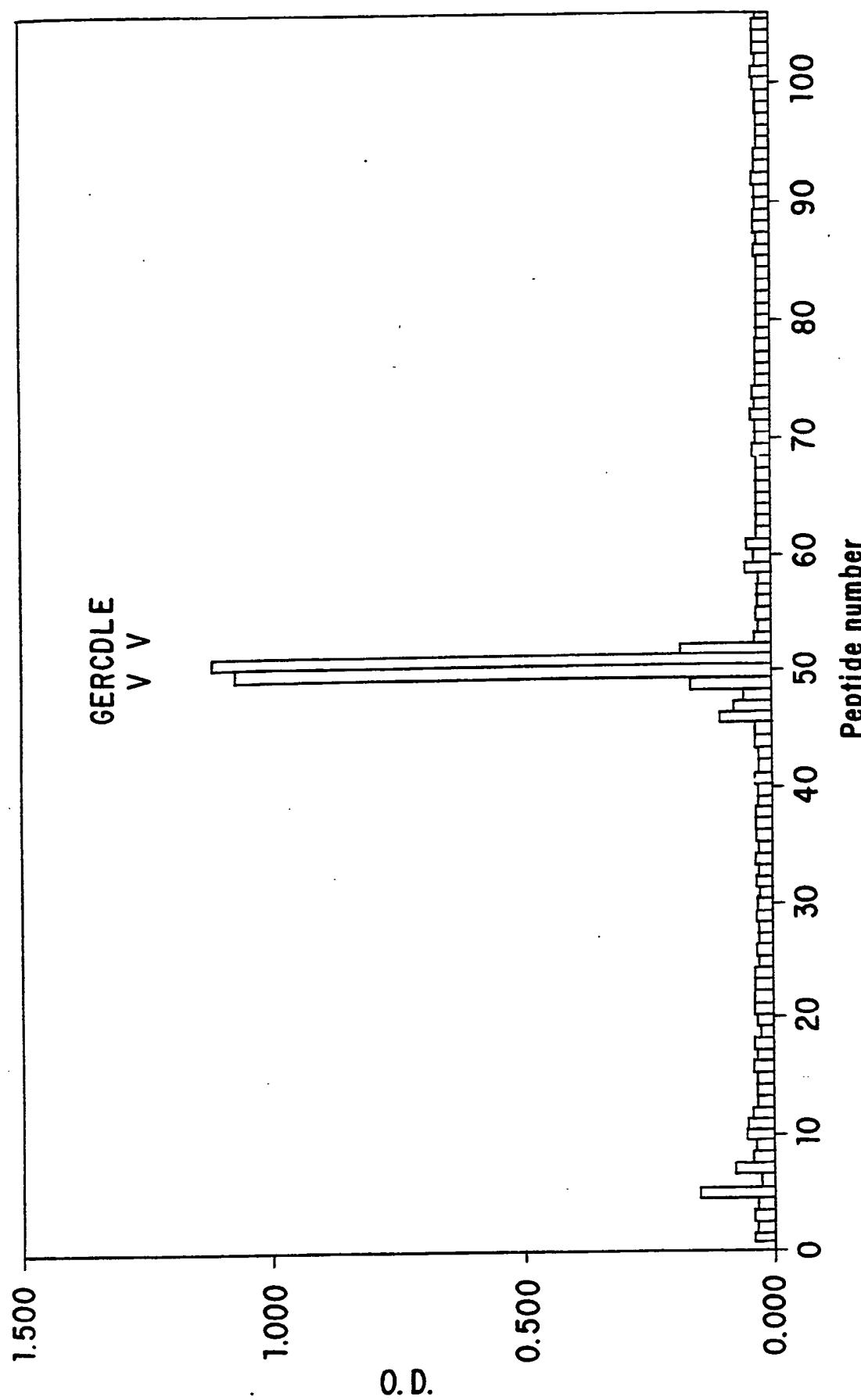


FIG. 3

4/4

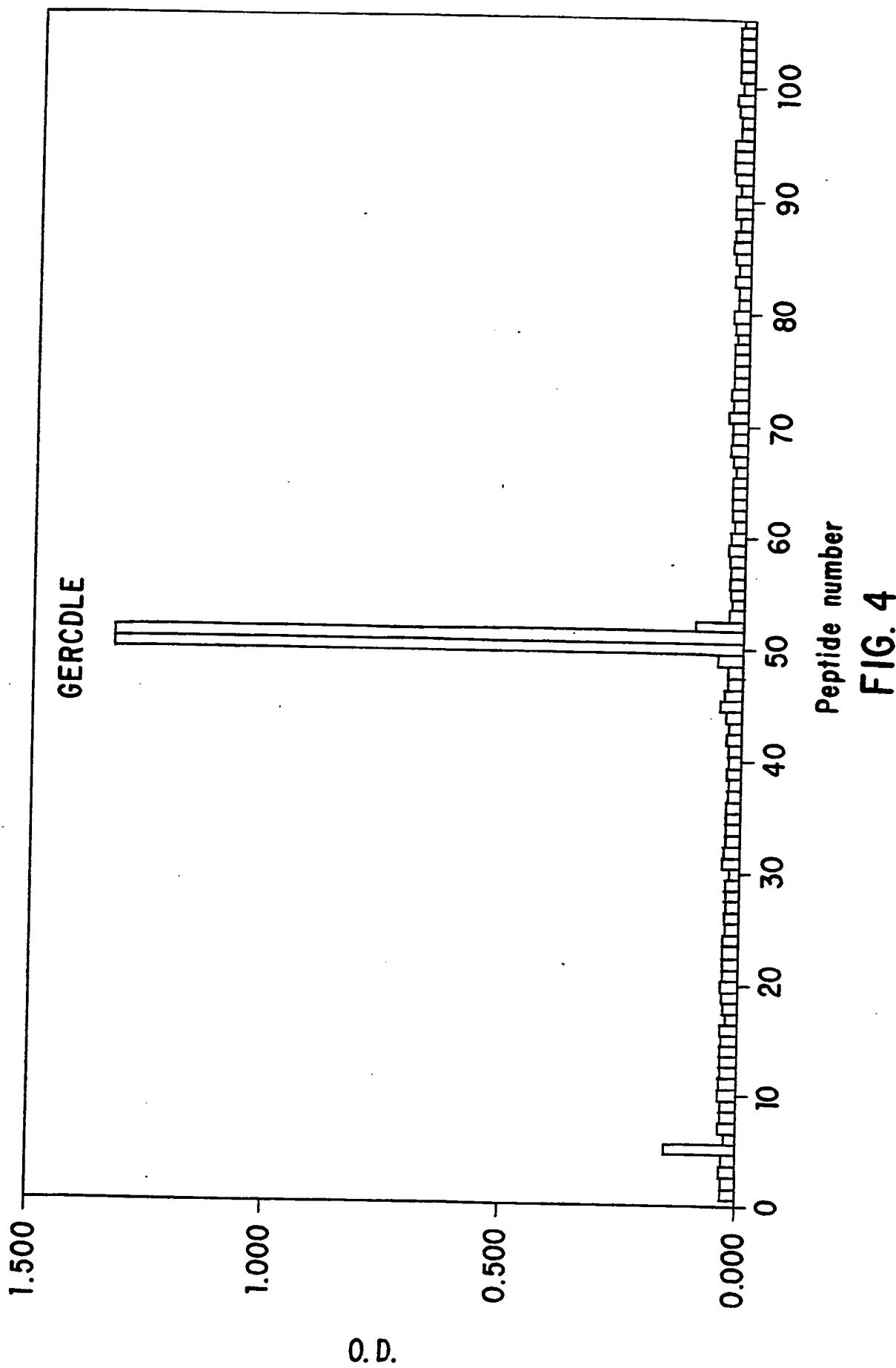


FIG. 4

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US92/07189

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.

US CL :435/5, 240.27; 530/388.3

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/5, 70.21, 172.2, 240.27; 530/388.3; 436/518, 548, 820

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US, A, 5,106,726 (Wang) 21 April 1992, Table 6; col. 5, lines 30-54; Example 8; Example 14; col. 6, lines 32-34; col. 28, line 50-col. 29, line 4.	1-10
Y	EP, A, 0,318,216 (Houghton et al.) 31 May 1989, page 20, lines 15-49.	11-29

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance		
"E" earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

18 November 1992

Date of mailing of the international search report

02 DEC 1992

Name and mailing address of the ISA/  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231  
Facsimile No. NOT APPLICABLE

Authorized officer

DONNA C. WORTMAN

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/07189

A. CLASSIFICATION OF SUBJECT MATTER:  
IPC (5):

C12Q 1/70; C12N 5/20; C07K 13/00